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Effect of lactic acid fermentation, boiling and soaking on selected nutrients and health promoting components of mango seed kernels

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Abstract

Mango seed kernels are considered as wastes although they are rich in essential nutrients and bioactive compounds for human health. Lack of commercial application (unlike oil seeds) presence of antinutrients, difficulty in processing and little information on nutritional and functional values contribute significantly to their underutilization. These factors underscore the need for processing these seeds to enhance their utilization as food or functional food. The purpose of this study was to investigate which processing technique was capable of improving selected nutrients and bioactive compounds, and reduction of the antinutritional factors to acceptable levels. Selected vitamins, minerals and antinutrients, antioxidant activity and total phenols were determined using standard methods. All the processing methods at different set conditions significantly ($p<0.05$) reduced the antinutritional factors of the mango seed kernels to above 38%. The results showed that, lactic acid fermentation had no significant differences in all analyzed minerals while boiling and soaking reduced the contents of the minerals except for potassium and zinc on soaked samples. The maximum percentage increase of total phenolic content, antioxidant activity and ascorbic acid was observed in samples fermented with *Lactobacillus plantarum* and their values were 25%, 37% and 28% respectively. On contrast, boiled and soaked samples had a significant decrease in ascorbic acid and antioxidant activity and all employed processing techniques showed insignifant variations of α-tocopherol content. The results in this study indicated that lactic acid fermentation reduced the antinutrients to acceptable levels and improved the studied nutritional and bioactive compounds as compared to boiling and soaking methods, thus considered as a technique for processing mango seed kernels for functional foods.

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Introduction

Plants naturally contain a mixture of compounds that may act independently or in combined form to improve the health of human (Lasano *et al.,* 2019). Recent studies have found higher levels of potential nutrients and bioactive compounds in waste food materials than in the actual products, comprising a wide category of fruits and seeds (Olawoye and Gbadamosi, 2017). Mango seed kernels are among the discarded parts of the fruits. These seeds are good source of phytochemical compounds, carbohydrate, fat, protein, vitamins, minerals and dietary fibers which possess a nutritional and protective effect against oxidative stress related diseases that are likely to compromise the health of human (Lasano *et al.,* 2019.) Despite the potential nutrients and bioactive compounds packed in these seeds, they are extensively disposed as wastes by consumers and fruit processors during the making of juice, jams and snacks. For example, there is about 3×10^5 tons of dry mango seed kernels not utilized annually in India after consumption or industrial processing of mango fruits (Soong and Barlow, 2004). Thus, it would be useful to improve the complete utilization of the mango seed kernels to be used as source of nutritional and functional values in food formulations.

The underutilization of mango seed kernels has been contributed by lack of commercial application (unlike oil seeds), lack of popularity, poor taste, difficulty in processing and little or lack of scientific information about their nutritional and functional properties hence pose a serious threat to the environment (Soong and Barlow, 2004; Lim *et al.,* 2019). Processing of mango seed kernels into food product to be incorporated in functional foods could be a way out of adding value to them and solving the environmental problem upon their disposal. It is also the best approach of creating income for all individuals who will be involved in the value chain. Additionally, the processed products from mango seed kernels can ultimately intensify the choice space of foodstuffs among the people. Nevertheless, currently there is no a processing method for reducing the antinutrients to acceptable levels and retaining or improving the nutrients and bioactive compounds of mango seed kernels. Most of the existing techniques have focused only in reducing or removing of the antinutritional factors without considering on the effects of other functional and essential nutrients such as heat sensitive and water soluble ones. For example the study conducted by Diarra (2014) on the potential of mango seed kernels on various methods focused only on the reduction of antinutrients while recommending further research for establishing optimal conditions that would prevent the loss of soluble nutrients during soaking and boiling methods. Therefore, the aim of this study was to explore which processing technique was capable of improving nutritional and bioactive compounds, and reduce the antinutritional factors to acceptable levels in mango seed kernels. We anticipate that the findings achieved in the present study will contribute additional utilization of mango seed kernels into added value products to tap their nutritional and health benefits.

Materials and methods

Sample preparation

Mango seed kernels were manually separated from matured and ripen mangoes of dodo variety by kitchen knife followed by being washed by clean water. The separated mango seeds kernels were immediately sliced crossectionally at 0.4×1cm thickness for each slice. The chosen thickness was within the range used by other scholars. For example Dorta *et al.* (2012) used the thickness of 0.5cm in drying treatments for stabilizing mango seed kernel on the effect of antioxidant activity. The sliced mango seed kernels were then placed into prepared containers ready for the soaking, lactic acid fermentation and boiling.

Processing methods

Lactic acid fermentation

The overall process of lactic acid fermentation employed three important phases. The first phase was the preparation of the starter cultures and samples. Lactic acid bacterial (LAB) that is *Lactobacillus johnsonii* (B-2178), *Lactobacillus rhammnosus* (B-

58149) and *Lactobacillus plantarum* (B-3058) were ordered from the Agricultural Research Service Culture Collection (NRRL) United States, Department of Agriculture, Peoria, Illinois, USA. All the starter cultures received were in a lyophilized state, thus activation process was needed in order for them to work properly. Therefore activation process of the strains was done in accordance to the method portrayed by Nyamete *et al.* (2016). The sliced pieces of mango seed kernels were initially immersed in ethanol followed by washing several times with distilled water before being inoculated so as to kill any foreign microorganism which could hamper the controlled fermentation. The second phase involved inoculating of the prepared samples with deionized water in the ratio of 2:2 by 1 ml of lactic acid bacteria. The inoculated samples were mixed well and covered aseptically, and then placed into the incubators set at recommended temperatures of the manufacture of the used strains (i.e. 30°C, 37°C and 42°C). The third phase encompassed observing of lactic acid fermentation by measuring parameters which included the pH and lactic acid before and during fermentation at an interval of 4 hours to completion of lactic acid fermentation within 24 hours. The pH was determined using a digital pH meter and the lactic acid was measured by calorimetric procedures as reported by Taylor (1996). After the lactic acid fermentation, the fermented samples were taken off from the incubators and put into the solar tunnel dryer till the drying was attained.

Soaking

Slice pieces of mango seed kernels were soaked in the distilled water at a ratio of 300g in 20 liters with varying time of 6 hours, 12 hours, 18 hours and 24 hours as adopted from Adeleke *et al.*(2017) with some modifications. Immediately after soaking, the sample where drained from water and dried into the solar dryer tunnel until the required moisture content was attained.

Boiling

Two liters of distilled water was dispensed into the water bath and then switched on up to boiling was achieved. The sliced pieces of mango seeds were placed into the boiling water in the water bath set at a constant temperature of 100°C. The samples put in the water bath were observed at a continuous boiling temperature of 100°C with different time interval of 5 minutes, 10 minutes, 15 minutes and 20 minutes as portrayed by Talabi *et al.* (2016) with some modifications. After each set time, the samples were removed from the water bath and dried by the solar dryer tunnel until the steady moisture content was attained.

Drying and grinding

The fermented, soaked and boiled samples were dried by a solar dryer tunnel until the required moisture content was attained. The dried mango seed kernels were then grounded by an electric blender, packed in the airtight containers and stored at room temperature waiting for analysis.

Analysis of antinutritional factors of the selected nutrients and bioactive compounds of raw and processed mango seed kernels

Anti-nutritional factors

The analyzed anti-nutrients involved the phytates, tannins, saponin and oxalates. Tannin was analyzed by using UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan) as reported by Arslan *et al.* (2016), whereas Saponin content was determine by the screening method as described by Ejikeme *et al.*(2014). Phytates and oxalates were determined by the HPLC as described by Vũ *et al.* (2013).

Determination of total phenolic compounds

Analysis of total phenolic compounds were measured by a modified technique of Molyneux (2004) and Arslan *et al.* (2016). Shortly, about 10 mg of the homogenized sample was extracted with 20ml of 50% aqueous methanol at 80°C for 2 hours, followed by filtration and the volume was made to about 50 ml. Then 1 ml of the solution was poured into a volumetric flask (50 ml) and 20 ml of distilled water added followed by 2.5 ml of folin-ciocalteu reagent and 10 ml of 17% Na_2CO_3. The blend was further homogenized and made to 50ml with distilled water

and after about 20 minutes, absorbance was read by UV spectrophotometer at 760 nm using gallic acid standard. The concentrations of total phenolics were calculated using the standard calibration curve of gallic acid and expressed as gallic acid equivalents per 100g.

Analysis of antioxidant activity using DPPH

Antioxidant activity was determined using a modified version of the method described Molyneux (2004) which is based on the principle of scavenging the DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical. About 0.5 ml of DPPH was mixed to the solutions prepared with 2.5ml of the homogenized sample and 3ml of methanol. The obtained blend was properly shaked for about 20 minutes and kept in the dark area for 2 hours. Similar procedure was done for the control sample. The UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan) was used to read the absorbance at 517nm. Ascorbic acid was used as a standard. There after the radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition of DPPH} = \{(A_B - A_A)/A_B\} \times 100 \quad \text{Eq.1}$$

Where A_B is the absorbance of the control sample, and A_A is the absorbance of the test extract solution. The results were expressed in IC_{50} values (concentration of sample required to scavenge 50% of free radicals) which were determined from a plot of % inhibition of DPPH versus concentration of the analyte.

Mineral determination

The minerals were determined using an atomic absorption spectrophotometer (AAS) as described by Nanda *et al.* (2003). Briefly, 5 g of the sample weighed was placed into a cleaned and dried crucible. The weighed crucible holding the sample was put into the muffle furnace which was set at 550°C temperatures. After complete ashing the crucible was removed from the muffle furnace and left to cool at room temperature. The obtained ash was transferred to a beaker (100mL) using 20.0mL of 1N of hydrochloric acid, then heated at 80-90°C for 5 minutes. After being heated, it was then transferred to a volumetric flask (100mL) and filled with 1N of hydrochloric acid to the mark, filtered to remove insoluble substances and the obtained analyte was kept in a labeled container waiting for analysis using an atomic absorption spectrophotometer. The mineral standards were prepared to make the calibration curve.

Ascorbic acid determination

Ascorbic acid content was determined by high performance liquid chromatography (HPLC) using UV-VIS detector of 254nm as portrayed by Vikram *et al.*(2005) with some modifications. Briefly, triplicate samples of about 2.5 g were weighed and extracted with 0.8% metaphosphoric acid as a mobile phase. The blend was made to 20mL followed by centrifugation at 10000rpm for about 20 minutes. The mixture was filtered with what man filter paper 1 and the collected analyte was again diluted with 10mL of 0.8% metaphosphoric acid. The obtained supernatant was again filtered through 0.45µ filter and the isolate was injected into the HPLC. Various concentrations of ascorbic acid standards were prepared for making a calibration curve.

Analysis of α-tocopherol

Vitamin E (α-tocopherol) was determined by a modified technique of Barker *et al.* (1998) using the HPLC. Approximately 2.5 g of the homogenized plant extract was blended with 4ml of 95% ethanol and 1ml of 50% potassium hydroxide. The blend were saponified by heating at 70°C in the water bath for about 20 minutes and there after left to cool in an ice bath vessel. Vitamins (Fat-soluble) were extracted with 1ml hexane containing 0.2% of butylated hydroxytoluene (BHT), and 1ml aliquot of the hexane layer was vaporized under nitrogen. Saponification, extraction and vaporization procedures were done under yellow light. There after the samples were reconstituted with 0.25 ml ethanol comprising 0.1% of butylated hydroxytoluene. Quantification of α-tocopherol content as a degree of vitamin E was made by a Shimadzu 20A Series liquid chromatograph equipped with a 250 × 4.0 mm stainless steel ODS

reversed- phase column. The mobile phase used was in the ratio of 96:4 (methanol: water) for α-tocopherol detection. The α-tocopherol was monitored at 285nm (Shimadzu SPD 20A) and the external standards were related to sample extracts for measuring of vitamin concentrations.

Statistical analysis

The obtained data were analyzed using R statistical package (R Development Core Team, Version 3.6.2 Vienna, Austria) for Analysis of Variance to determine the significant ($p<0.05$) variations between the processing techniques. The means of analyzed total phenols, selected minerals and vitamins were separated by Turkey's Honest Significant difference (THSD) at $p<0.05$.

Results and discussion

Antinutrients

Mango seed kernels contain various antinutrients which complexes essential nutrients thus decreasing their bioavailability (Fowomola, 2010). Reducing or eliminating these antinutrients is needed to prevent poisoning and improving the biological utilization of mango seed kernels. In this study, the effect of lactic acid fermentation, soaking and boiling on the antinutrients reduction in mango seed kernels is being shown in Table 1.

In the analyzed antinutritional factors, tannins were observed to have the lowest content (0.49mg/100g) while phytates had the highest content (2.91mg/100g) in the raw mango seeds.

Table 1. Effects of soaking, boiling and lactic acid fermentation on the antinutrients reduction in mango seed kernels.

	Treatment	Tannins (mg/100g)	Oxalates (mg/100g)	Phytates (mg/100g)	Saponin
Boiling	Raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.49^{a}	+
	5 minutes	0.30±0.02^{b}	0.33±0.06^{b}	1.41±0.19^{b}	nd
	10 minutes	0.29±0.01^{b}	0.18±0.01^{c}	1.35±0.05^{b}	nd
	15 minutes	0.23±0.02^{c}	0.17±0.00^{c}	0.10±0.01^{c}	nd
	20 minutes	0.17±0.02^{d}	nd	0.06±0.00^{c}	nd
Soaking	Raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.29^{a}	+
	6 hours	0.06±0.00^{b}	0.05±0.01^{b}	1.58±0.08^{b}	nd
	12 hours	0.05±0.00^{b}	0.04±0.00^{b}	1.47±0.06^{b}	nd
	18 hours	nd	0.04±0.00^{b}	0.69±0.04^{c}	nd
	24 hours	nd	0.04±0.00^{b}	0.51±0.03^{c}	nd
Fermen't (30°C)	raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.49^{a}	+
	B-3058	0.29±0.00^{b}	0.36±0.04^{b}	0.47±0.02^{b}	nd
	B-2178	0.21±0.02^{c}	0.25±0.04^{c}	0.93±0.02^{bc}	nd
	B-59149	0.31±0.01^{b}	0.18±0.02^{d}	0.28±0.01^{c}	nd
Fermen't (37°C)	raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.45^{a}	+
	B-3058	0.20±0.07^{c}	0.18±0.01^{c}	0.20±0.02^{c}	nd
	B-2178	0.24±0.02^{b}	0.21±0.01^{b}	1.11±0.21^{b}	nd
	B-59149	0.03±0.00^{d}	0.07±0.01^{d}	0.16±0.00^{c}	nd
Fermen't (42°C)	raw	0.49±0.01^{a}	0.67±0.01^{a}	2.91±0.49^{a}	+
	B-3058	0.28±0.01^{b}	0.03±0.00^{c}	1.75±0.09^{b}	nd
	B-2178	0.03±0.00^{c}	0.05±0.00^{b}	1.71±0.07^{b}	nd
	B-59149	0.03±0.00^{c}	0.03±0.00^{c}	1.67±0.05^{b}	nd

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii,* 59149= *Lactobacillus rhammnosus* and Fermen't=Fermentation.

All processing techniques significantly (P<0.5) reduced the analyzed antinutritional factors in the mango seed kernels. Reduction of phytates, oxalates and tannins increased as time increased for boiling and soaking. Similar results for the reduction of antinutrients with increase in time length for boiling and soaking was observed in other findings reported by (Sotelo *et al.,* 2010).

Table 2. Effects of soaking, boiling and lactic acid fermentation on the selected vitamins, total phenol and antioxidant activity in mango seed kernels.

	Treatment	Ascorbic acid (mgAAE/100g)	α-tocopherol (mg/100g)	Total phenol (mgGAE/g)	DPPH radical IC_{50} (mg/ml)
Boiling	Raw	0.68±0.02^{a}	1.28±0.05^{a}	70.68±1.23^{c}	2
	5 minutes	0.19±0.04^{b}	1.13±0.02^{a}	87.21±6.95^{b}	2
	10 minutes	0.14±0.01^{bc}	1.14±0.05^{a}	88.15±1.27^{ab}	2.05
	15 minutes	0.11±0.01^{cd}	1.02±0.00^{a}	90.09±0.23^{a}	2.15
	20 minutes	0.05±0.00^{d}	1.04±0.02^{a}	90.78±0.84^{a}	2.25
Soaking	Raw	0.68±0.02^{a}	1.28±0.05^{a}	70.68±1.23^{a}	2
	6 hours	0.23±0.02^{b}	1.17±0.15^{a}	69.66±2.30^{a}	2.01
	12 hours	0.16±0.01^{b}	1.23±0.04^{a}	68.98±1.07^{ab}	2.25
	18 hours	0.07±0.01^{c}	1.20±0.01^{a}	68.34±0.28^{ab}	2.3
	24 hours	0.06±0.00^{c}	1.10±0.06^{a}	62.75±3.41^{b}	2.4
Fermen't (30°C)	Raw	0.68±0.02^{a}	1.28±0.05^{a}	70.68±1.23^{b}	2
	B-3058	0.70±0.03^{a}	1.34±0.02^{a}	82.86±1.01^{a}	1.55
	B-2178	0.60±0.04^{b}	1.40±0.02^{a}	80.71±7.27^{a}	1.55
	B-59149	0.54±0.01^{c}	1.31±0.01^{a}	80.16±1.40^{a}	1.8
Fermen't (37°C)	Raw	0.68±0.02^{ab}	1.28±0.05^{a}	70.68±1.23^{c}	2
	B-3058	0.95±0.02^{a}	1.33±0.03^{a}	94.84±6.73^{a}	1.25
	B-2178	0.64±0.01^{ab}	1.29±0.07^{a}	92.82±1.41^{a}	1.5
	B-59149	0.49±0.09^{b}	1.15±0.07^{a}	86.49±0.23^{b}	1.3
Fermen't (42°C)	Raw	0.68±0.02^{a}	1.28±0.05^{a}	70.68±1.23^{b}	2
	B-3058	0.60±0.03^{ab}	1.41±0.04^{a}	87.69±3.90^{a}	1.8
	B-2178	0.57±0.05^{b}	1.24±0.02^{a}	85.54±3.68^{a}	1.55
	B-59149	0.55±0.0^{b}	1.34±0.01^{a}	75.68±6.95^{b}	1.61

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different (p<0.05); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii,* 59149= *Lactobacillus rhammnosus* and Fermen't=Fermentation.

The reasons for increase reduction of antinutrients with time increase could be due to more leaching and diffusion of the antinutrients into water as time increased. No saponin content was detected at all set conditions after boiling, lactic acid fermentation and soaking of the mango seed kernels. With lactic acid fermentation reduction of antinutrients for tannins and oxalates was more than 55% at both 37°C and 42°C. It was also observed that maximum reduction (more than 60%) of tannins and oxalates with boiling was achieved at 20 minutes while soaking reduced tannins and oxalates for more than 86% from 6 hours to above. The reduction observed in the antinutrients of mango seeds kernels had been reported in other previous studies (Torres-León *et al.,* 2016 ; Adegbehingbe *et al.,* 2017).

Table 3. Effects of soaking, boiling and lactic acid fermentation on the analyzed minerals in mango seed kernels (mg/100g).

	Treatment	Calcium	Sodium	Potassium	Iron	Zinc	
Boiling	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}	
	5 minutes	118.02±1.91^{a}	258.21±0.19^{b}	289.54±3.14^{a}	4.13±0.06^{b}	0.51±0.01^{b}	
	10 minutes	117.40±1.38^{a}	254.74±6.18^{bc}	285.83±10.84^{ab}	4.02±0.08^{b}	0.51±0.02^{b}	
	15 minutes	103.63±4.28^{b}	247.96±1.48^{cd}	272.54±7.28^{b}	3.57±0.09^{c}	0.50±0.01^{b}	
	20 minutes	101.77±2.17^{b}	243.45±0.28^{d}	249.70±2.70^{c}	3.25±0.10^{d}	0.48±0.03^{b}	
Soaking	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}	
	6 hours	120.01±3.61^{a}	273.36±1.90^{b}	299.53±2.09^{a}	4.27±0.07^{b}	0.60±0.10^{a}	
	12 hours	117.51±2.42^{ab}	235.07±1.37^{b}	297.23±3.25^{a}	3.68±0.10^{c}	0.57±0.02^{a}	
	18 hours	111.97±1.4^{4b}	234.83±1.49^{b}	296.69±2.32^{a}	3.36±0.16^{d}	0.54±0.08^{a}	
	24 hours	98.08±0.76^{c}	234.39±1.16^{c}	287.39±0.65^{a}	2.99±0.08^{e}	0.50±0.02^{a}	
Fermen't (30°C)	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}	
	B-3058	119.96±1.28^{a}	286.92±3.80^{a}	296.87±4.96^{a}	4.63±0.06^{a}	0.60±0.07^{a}	
	B-2178	122.34±0.69^{a}	288.70±8.46^{a}	299.29±7.50^{a}	4.63±0.06^{a}	0.69±0.02^{a}	
	B-59149	121.77±0.66^{a}	287.95±10.28^{a}	292.44±10.40^{a}	4.54±0.01^{a}	0.64±0.06^{a}	
Fermen't (37°C)	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}	
	B-3058	121.86±3.22^{a}	277.92±6.96^{a}	301.44±1.14^{a}	4.76±0.05^{a}	0.57±0.04^{a}	
	B-2178	120.87±3.87^{a}	279.26±7.77^{a}	303.95±2.12^{a}	4.66±0.11^{a}	0.60±0.01^{a}	
	B-59149	122.51±8.93^{a}	280.45±6.72^{a}	304.78±0.59^{a}	4.62±0.01^{a}	0.60±0.01^{a}	
Fermen't (42°C)	Raw	120.77±0.87^{a}	275.28±2.34^{a}	301.45±2.57^{a}	4.70±0.12^{a}	0.64.±0.05^{a}	
	B-3058	122.25±2.53^{a}	276.33±1.96^{a}	301.29±6.19^{a}	4.70±0.10^{a}	0.62±0.04^{a}	
	B-2178	120.08±0.73^{a}	276.79±7.82^{a}	303.10±2.95^{a}	4.53±0.05^{a}	0.64±0.03^{a}	
	B-59149	121.66±0.74^{a}	272.02±6.46^{a}	303.65±3.88^{a}	4.51±0.14^{a}	0.64±0.01^{a}	

Data presented as means ±SD (n=3). The means in columns with different superscript letters are significantly different ($p<0.05$); B-3058= *Lactobacillus plantarum*, B-2178= *Lactobacillus johnsonii* and 59149= *Lactobacillus rhammnosus*.

This reduction in the anti-nutrients had been attributed by sensitivity to heat during boing, action of microorganisms during fermentation and leaching upon soaking (Azim *et al.,* 2007). The content of the antinutrients obtained in this study was a bit lower as compared to the findings published by Adegbehingbe *et al.* (2017) and Fowomola (2010). The difference in the contents of antinutrients obtained in both studies may be attributed to differences in cultivars of the mango seed kernels studied and experimental procedures used.

Vitamins (ascorbic acid and α-tocopherol), total phenol and antioxidant activity

The effects of lactic acid fermentation, soaking and boiling on the selected vitamins, total phenol and antioxidant activity in mango seed kernels is presented in Table 2.

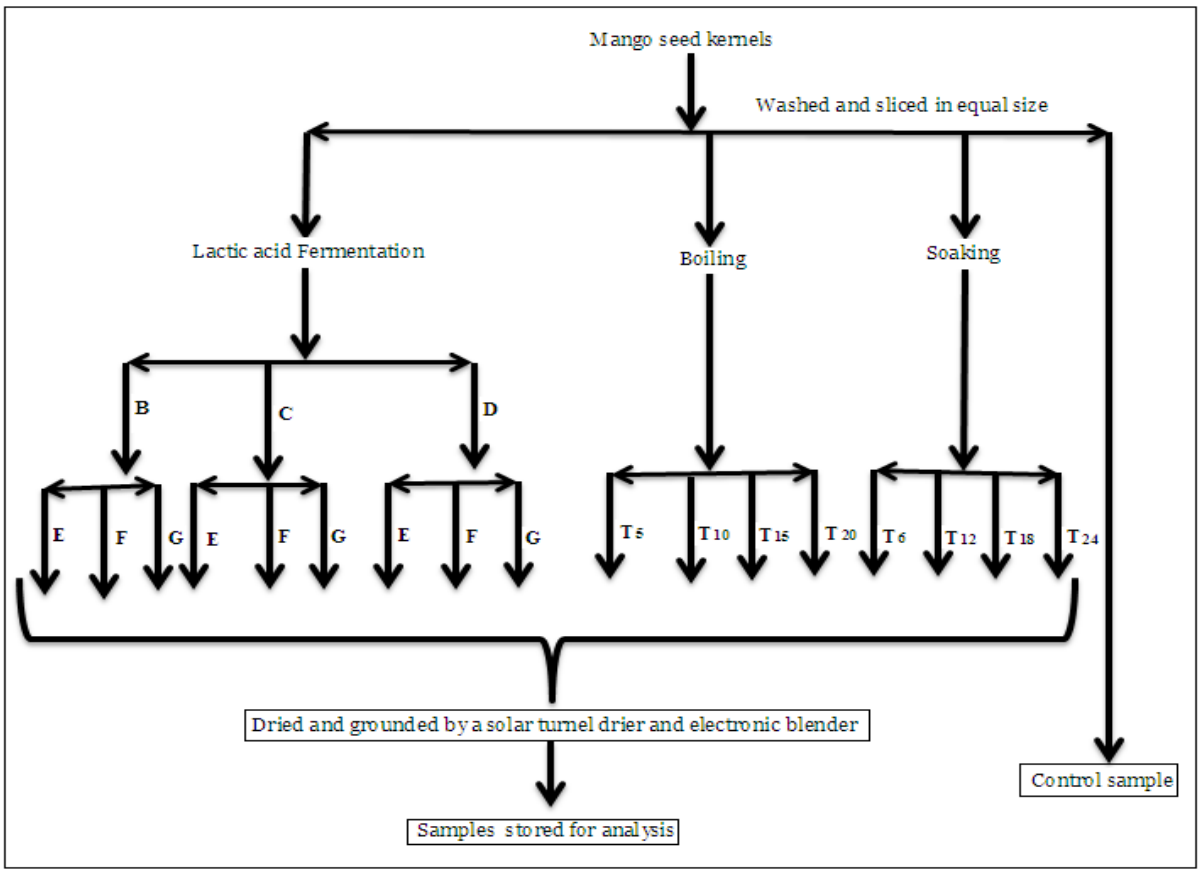

Fig. 1. Flow chart showing the experimental design with the major steps in the processing techniques. B= *Lactobacillus plantarum*, C= *Lactobacillus johnsonii*, D= *Lactobacillus rhammnosus*, E=30°C, F=37°C, G=42°C, T_5=Five minutes, T_{10}=ten minutes, T_{15} =fifteen minutes, T_{20}=Twenty minutes, T_6=six hours, T_{12}=Twelve hours, T_{18}=Eighteen hours and T_{24}= Twenty four hours.

Among the three processing techniques employed in this study, boiling and soaking reduced the content of ascorbic acid and the reduction increased with the increase of time. The decrease in ascorbic acid ranged from 0.68-0.06mgAAE/100g with soaking while boiling reduced the content of ascorbic acid from 0.68-0.05mgAAE/100g.

In all set conditions for boiling and soaking reduction of ascorbic acid content was higher than 66%. A significant reduction ($P<0.05$) in the ascorbic acid in mango seed kernels as a results of boiling and soaking was also reported in other previous published work. For example, it was reported by Sahlin *et al.* (2004) a loss of 24% ascorbic acid in two cultivars of tomatoes when boiled.

A similar decline in ascorbic acid content during soaking has been observed previously studies in soaked soy bean as reported by Kaushik and Satya (2010). The loss of ascorbic acid during boiling and soaking was due to the fact that ascorbic acid is a water soluble and heat sensitive vitamin (Okmen and Bayindirli, 1999). On the other hand, the result of lactic acid fermentation showed a significant decrease in ascorbic acid in some fermented samples except for samples fermented by *Lactobacillus plantarum* at 30°C and 37°C which both had an increased content of ascorbic acid.

The significant increase of ascorbic acid in mango seed kernel on lactic acid fermented samples by *Lactobacillus plantarum* at 30°C and 37°C were 0.70mgAAE/100g and 0.95mgAAE/100g respectively. This increase in ascorbic acid might be due to increased activities of fermenting microorganisms as observed in other findings (Adetuyi and Ibrahim, 2014; Jhan *et al.*, 2015).

All processing methods employed in this study showed no significant variation of α-tocopherol. Vitamin E (α-tocopherol) content of raw mango seed kernels was 1.28 mg/100g. Similar results within this range was reported by Fowomola (2010). However, the insignificant results observed in this study was contributed by inactivation of lipoxygenase enzyme responsible for lipid peroxidation upon boiling and the nature of vitamin E as a fat soluble vitamin hence not leached or dissolved in water during fermentation, boiling and soaking (Kansson and Jagerstad, 1990).

The total phenolic content of raw mango seed kernels was found to be 70.68mgGAE/g. The obtained results were much higher than that reported for seed kernels of other mango cultivars (Abdel-Aty *et al.,* 2018). Soaked mango seed kernels showed a significant reduction ($p<0.05$) in total phenol while lactic acid fermentation and boiled samples presented a significant increase of total phenol. The decline in bioavailability of total phenol in soaked mango seed kernels was attributed by leaching and diffusion of phenolics in cell liquids and such decrease was also seen in soaked green gram (Afify *et al.*, 2012; Oghbaei and Prakash, 2017).

The maximum reduction of total phenol in the soaked mango seed kernel was 11.2% and it was observed at 24 hours. Contrary results were observed with lactic acid fermented and boiled samples. The increase of total phenol in the lactic acid fermented and boiled mango seed kernels ranged from 70.68-94.84mgGAE/g and 70.68-90.78mgGAE/g respectively where the highest (24.16%) increase in total phenol content was observed at a fermentation temperature of 37°C in sample fermented by *Lactobacillus plantarum*. In normal form, phenolic compounds are mutual or bound with sugar which decreases their bio-availability. During lactic acid fermentation, proteolytic enzymes from the starter organism hydrolyse complexes of phenolics into soluble free phenols (Adetuyi and Ibrahim, 2014). Therefore, the increased content of total phenol was attributed by opening of the cell matrix during boiling and cleavage of the ester linkages by synthesizing enzymes in lactic acid fermentation (Acosta-Estrada *et al.*, 2014 ;Tian *et al.*, 2016).

As shown in Table 2, the DPPH radical scavenging activity of the mango seed kernels was expressed by IC_{50} values which is the concentration of antioxidant activity required to reduce the initial DPPH concentration by 50%. The Lower IC_{50} value indicates higher antioxidant activity (Olawoye and Gbadamosi, 2017). The results of scavenging DPPH with IC_{50} values of mango seed kernel ranged from 1.25 to 2.5mg/ml. In all set conditions for the processing techniques employed in this study, 1.25 mg/ml (the lowest IC_{50} value) was the highest antioxidant activity of the mango seed kernels. The loss of antioxidant activity was observed in both boiled and soaked mango seed kernels. The highest reduction of antioxidant activity in both boiled and soaked mango seed kernels were remarked at 20 minutes and 24 hours respectively. This reduction of the antioxidant activity was due to degradation of the phytochemicals and leaching of the essential antioxidants during boiling and soaking (Perla *et al.*, 2012). Lactic acid fermentation presented a significant increase in the antioxidant activity and the highest percentage (41.5%) was noted with sample fermented with *Lactobacillus plantarum*.

The observed increase in the antioxidant activity as a result of lactic acid fermentation reported in this study is in agreement with the data given by Virtanen *et al.,* (2007) who found that fermentation of milk whey using lactic acid bacteria resulted into an increase of its antioxidant activity. In addition, the extent of increased antioxidant activity of fermented mango seed kernel varied with the microorganisms used. Similar variations of antioxidant activities were observed when different starter microorganisms used in fermentation of soy milk (Wang *et al.,* 2006) Additionally, the high antioxidant activity observed as a result of lactic acid fermented samples could be due to the increased in hydroxyl groups or amino groups in the antioxidant compounds and synthesis of the phenolics (Olawoye and Gbadamosi, 2017).

Minerals

In Table 3, the mineral contents of raw, fermented, boiled and soaked mango seed kernels are presented. Minerals can be categorized into major and trace elements subject to their concentration present and amount required by human body (Lasano *et al.,* 2019). In the current study, three major minerals and two trace minerals were analyzed in mango seed kernels. The mineral contents (calcium, potassium, Iron and zinc) of mango seed kernel obtained in this study are a bit lower compared to those presented by Yatnatti *et al.* (2014).

This variation of mineral contents of the mango seed kernels might be due to the type of soil used, environmental conditions and mango cultivars. The results showed that, lactic acid fermentation had no significant differences in the analyzed minerals (calcium, sodium, potassium, iron and zinc) though there were some apparent differences detected among the samples in the treatments. Similar results were reported by Afoakwa *et al.* (2013) who noted insignificant change in sodium content of cocoa beans upon fermentation. Also Bilgiçli *et al.* (2006) observed no significant difference in calcium, potassium and Magnesium on fermented tarhana dough. However, boiling significantly reduced minerals (calcium 120.8-101.8mg/100g, sodium 275.3-243.5mg/100g, potassium 301.5-249.7mg/100g, iron 4.7-3.3mg/100g and zinc 0.64-0.5mg/100g) while soaking reduced the components of some analyzed minerals (calcium 120.8-98mg/100g, sodium 275.3-234.4mg/100g and iron 4.7-3 mg/100g).

The decline in mineral contents in soaked and boiled samples was in agreement with other scholars (Hefnawy, 2011; Ojha *et al.,* 2020). Of all the minerals analyzed, the highest reduction (36%) was observed in soaked sample at 24 hours in iron whereas the lowest reduction (15.7%) was noted in boiled sample at 20 minutes in calcium. The significant reduction of some minerals in boiled and soaked samples was due to leaching in water during processing (Hefnawy, 2011).

In general, lactic acid fermentation method demonstrated good findings in terms of reducing the antinutrients to acceptable levels, improving or retaining of the selected nutrients and bioactive components of the processed mango seed kernels compared to soaking and boiling techniques.

This was explained by the observed incremental or insignificant change in contents of the analyzed ascorbic acid, minerals, total phenolics, antioxidant activity and α-tocopherol, and reduced or eliminated contents of the antinutritional factors to acceptable levels as shown in Table 1, 2 and 3.

The reasons that contributed for lactic acid to have good results were due to the capability of the used strains to synthesize and disrupt the ester bonds, suitable substrate and utilization of the substrate by lactic acid bacteria. With these observed facts in this study, lactic acid fermentation technique qualifies to process mango seed kernels for functional foods.

Conclusion

The use of lactic acid fermentation technique in processing mango seed kernels has played a great role over the boiling and soaking methods in reducing the antinutrients to acceptable levels and retaining or improving the selected nutrients and bioactive compounds which all have benefits to human health.

Therefore, with these evidences it is clear that lactic acid fermentation is an opted method for processing mango seed kernel to be incorporated in functional foods. We recommend further studies to be conducted to analyze and identify components which have contributed either to increase or retention of the total phenolics and antioxidant activity. Also clinical researches should be conducted to reveal these promising results.

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Conflict of interest

No conflict of interest was declared by the authors.

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